Application No.: 10/031,695
Inventor: HAUER et al.
Reply to Office Actions of 30 November 2005
26 April 2006 & 20 June 2006
Docket No.: 51241

Amendments to the Claims:

1. (withdrawn) A modified cytochrome P450 monooxygenase which, in comparison with the wild-type enzyme, shows an altered substrate profile in the terminal and/or subterminal enzymatic hydroxylation of aliphatic carboxylic acids, owing to site-specific mutagenesis of its substrate binding region.

2. (withdrawn) A monooxygenase as claimed in claim 1, which is derived from cytochrome P450 monooxygenases of bacterial origin.

3. (withdrawn) A monooxygenase as claimed in claim 2, which is derived from *Bacillus megaterium* cytochrome P450 monooxygenase BM-3 with an amino acid sequence in accordance with SEQ ID NO:2, which has at least one functional mutation in one of the following amino acid sequence regions: 24-28, 45-51, 70-72, 73-82, 86-88, 172-224 and 352-356, with the proviso that, if the enzyme carries the mutation FS7A, more than one of these regions is mutated.

4. (withdrawn) A monooxygenase as claimed in claim 3, which comprises at least one functional mutation in the amino acid sequence regions 86-88 and 172-224.

5. (withdrawn) A monooxygenase as claimed in claim 4, which comprises at least one of the following amino acid substitution patterns:
   a) F87V;
   b) F87A L188K;
   c) F87V L188K;
   d) F87A L188 KA74G;
   e) F87V L188K A74G;
   f) F87A L188K A74G R47F;

g) F87V L188K A74G R47F;

h) F87A L188K A74G R47F V26T; or

i) F87V L188K A74G R47F V26T;

and functional equivalents thereof.

6. (withdrawn) A monooxygenase as claimed in claim 3, which comprises a single amino acid substitution from amongst the following:

a) V26T,

b) R47F,

c) S72G,

d) A74G,

e) F87V,

f) L188z, where Z is an amino acid selected from amongst K, R, W, Q, N, G, A and S, and

g) M354T;

and functional equivalents thereof.

7. (withdrawn) A nucleic acid sequence encoding a monooxygenase as claimed in claim 1 and the complementary nucleic acid sequence thereof.

8. (withdrawn) An expression construct comprising, under the genetic control of regulatory acid sequence, an encoding sequence which encompasses a nucleic acid sequence as claimed in claim 7.

9. (withdrawn) A vector which encompasses at least one expression construct as claimed in claim 8.

10. (withdrawn) A recombinant microorganism which has been transformed with at least one

vector as claimed in claim 9.

11. (withdrawn) A microorganism as claimed in claim 10, selected from amongst bacteria of the genus *Escherichia*.

12. (currently amended) A process for the enzymatic production of subterminally hydroxylated aliphatic carboxylic acids, which comprises

a1) culturing a recombinant microorganism which has been transformed with a vector which encompasses an expression construct comprising, under the genetic control of regulatory nucleic acid sequences, a sequence which encompasses a nucleic acid sequence encoding a monooxygenase which is derived from *Bacillus megaterium* cytochrome P450 monooxygenase BM-3 with an amino acid sequence ~~in accordance with~~ containing SEQ ID NO:2 which has a functional mutation in the amino acid sequence region 86-88 and optionally at least one further functional mutation in one of the fo11owing amino acid sequence regions: 24-28, 45-51, 73-82 and 172-224 with the proviso that, if the enzyme carries mutation F87A, more than one of these regions is mutated, which functional mutation in comparison with the wild-type enzyme, results in an altered activity or regioselectivity in the subterminal enzymatic hydroxylation of [[,]] an aliphatic C_8-C_{12}-carboxylic acid, whereby culturing is performed in the presence of a culture medium which contains at least one hydroxylatable C_8-C_{12}-carboxylic acid or a derivative thereof, said derivative being selected from ~~at~~ an alkyl ester, an amide or an anhydride ~~thereof~~ of the at least one hydroxylatable C_8-C_{12}-carboxylic acid; or

a2) incubating a reaction medium containing at least one hydroxylatable C_8-C_{12}-carboxylic acid or a derivative thereof, said derivative being selected from an alkyl ester, an amide or an anhydride ~~thereof~~ of the at least one hydroxylatable

C_8-C_{12}-carboxylic acid with a modified monooxygenase as defined above, and

b) isolating the resulting hydroxylated product from the medium.

13. (canceled)

14. (currently amended) A method as claimed in claim 12, wherein the at least one hydroxylatable carboxylic acid is a C_8-C_{12}-monocarboxylic acid or [[a]] the derivative thereof, and the monooxygenase comprises at least one of the following amino acid substitution patterns in an amino acid sequence ~~according to~~ of SEQ ID NO:2:

a) F87V;

b) F87A and L188K;

c) FS7V and L188K

d) F87A L188K and A74G;

e) F87V L188K and A74G;

f) F87A L188K ~~and~~ A74G and R47F;

g) F87V L188K ~~and~~ A74G and R47F;

h) F87A L188K A74G R47F and V26T; or

i) F87V L188K A74G R47F and V26T.

15. (canceled)

16. (previously presented) A method as claimed in claim 12, wherein the enzymatic production is carried out in the presence of an electron donor or a reduction equivalent.

17. (currently amended) A method as claimed in claim 16, wherein the electron donor or the reduction equivalent is selected from ~~amongst~~ the group consisting of NADH, NADPH and Zn/CO(III) sepulchrate.

18. (currently amended) The process of claim 12, wherein the monooxygenase comprises at least one functional mutation in the amino acid sequence of regions 86-88 and at least one functional mutation in the amino acid sequence of region 172-224.

19. (cancelled)